

AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE SPECIFICATION:

Kindly amend the claims, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, to read as follows:

Kindly amend the paragraph at page 2, lines 28 to 35, to read as follows:

In ~~June 1998~~ June 1998, Cole et al published the complete genome sequence of *M. tuberculosis* and predicted the presence of approximately 4000 open reading frames (Cole et al 1998). Following the sequencing of the *M. tuberculosis* genome, nucleotide sequences comprising Rv2653c, Rv2654c and Rv3873 are described in various databases and putative protein sequences for the above sequences are suggested, Rv2653c either comprising methionine or leucine as the first amino acid (The Sanger Centre database (~~http://www.sanger.ac.uk/Projects/M_tuberculosis~~), Institut Pasteur database (~~http://genolist.pasteur.fr/TubercuList~~) and GenBank (~~http://www4.ncbi.nlm.nih.gov~~ at the National Center for Biotechnology Information database maintained by the National Institutes of Health)).

Kindly amend the paragraph at page 13, lines 15 to 29, to read as follows:

The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. The two sequences to be compared must be aligned to best possible fit possible with the insertion of gaps or alternatively, truncation at the ends of the protein sequences. The sequence identity can be calculated as $\frac{(N_{ref} - N_{dif})}{N_{ref}} \times 100$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ($N_{dif}=2$ and $N_{ref}=8$). A gap is counted as non-identity of the specific

residue(s), i.e. the DNA sequence AGTGTC will have a sequence identity of 75% with the DNA sequence AGTCAGTC ($N_{\text{dif}}=2$ and $N_{\text{ref}}=8$). Sequence identity can alternatively be calculated by the BLAST program e.g. the BLASTP program (Pearson W.R and D.J. Lipman (1988)) (~~www.ncbi.nlm.nih.gov/cgi-bin/BLAST~~ available at the National Center for Biotechnology Information website as maintained by the National Institutes of Health). In one aspect of the invention, alignment is performed with the sequence alignment method ClustalW with default parameters as described by Thompson J., *et al* 1994, available at <http://www2.ebi.ac.uk/clustalw/> the European Bioinformatics Institute website.

Kindly amend Table 1 starting on page 30, line 11, to read as follows:

Table 1. Sequence of the *rd1-orf5* oligonucleotides^a.

| Orientation and oligo-nucleotide | Sequences (5'→ 3') | Position (nt) |
|----------------------------------|---|---------------|
| <hr/> | | |
| Sense | | |
| RD1-ORF5f | <u>CTGGGGATCCGCGTGATCACCAT-</u> GCTGTGG <u>SEQ ID NO: 67</u> | 3028 - 3045 |
| Antisense | | |
| RD1-ORF5r | <u>TGCAAGCTTTCACCAGTCGTCCT-</u> CTTCGTC <u>SEQ ID NO: 68</u> | 4243 - 4223 |

^a The oligonucleotides were constructed from the Accession number U34484 nucleotide sequence (Mahairas et al., 1996). Nucleotides (nt) underlined are not contained in the nucleotide sequence of RD1-ORF5. The positions correspond to the nucleotide sequence of Accession number U34484.

Kindly amend the paragraph at page 36, lines 2 to 42, to read as follows:

Peptide synthesis: The immunological evaluation of recombinant RD1-ORF5 was described in example 2. Thirty-five overlapping peptides covering the complete amino acid sequence of RD1-ORF5 were purchased from Mimotopes Pty Ltd. The peptides were synthesized by Fmoc solid phase strategy. No purification steps were performed. Lyophilised peptides were stored dry until reconstitution in PBS.

RD1-ORF5-p1 MDYFIRMWNQAALAMEVY SEQ ID NO: 23

| | | |
|--------------|----------------------|----------------------|
| RD1-ORF5-p2 | AALAMEVYQAETAVNTLF | <u>SEQ ID NO: 24</u> |
| RD1-ORF5-p3 | ETAVNTLF EKLEPMASIL | <u>SEQ ID NO: 25</u> |
| RD1-ORF5-p4 | LEPMASILDPGASQSTTN | <u>SEQ ID NO: 26</u> |
| RD1-ORF5-p5 | GASQSTTNPIFGMPSPGS | <u>SEQ ID NO: 27</u> |
| RD1-ORF5-p6 | FGMPSPGSSTPVGQLPPA | <u>SEQ ID NO: 28</u> |
| RD1-ORF5-p7 | PVGQLPPAATQTLGQLGE | <u>SEQ ID NO: 29</u> |
| RD1-ORF5-p8 | QTLGQLGEMSGPMQQLTQ | <u>SEQ ID NO: 30</u> |
| RD1-ORF5-p9 | GPMQQLTQPLQQVTSLSFS | <u>SEQ ID NO: 31</u> |
| RD1-ORF5-p10 | QQVTSLSFSQVG GTGGGNP | <u>SEQ ID NO: 32</u> |
| RD1-ORF5-p11 | GGTGGGNPADEEAAQMGL | <u>SEQ ID NO: 33</u> |
| RD1-ORF5-p12 | EEAAQMGLLGTSPLSNHP | <u>SEQ ID NO: 34</u> |
| RD1-ORF5-p13 | TSPLSNHPLAGGSGPSAG | <u>SEQ ID NO: 35</u> |
| RD1-ORF5-p14 | GGSGPSAGAGLLRAESLP | <u>SEQ ID NO: 36</u> |
| RD1-ORF5-p15 | LLRAESLPGAGGSLTRTP | <u>SEQ ID NO: 37</u> |
| RD1-ORF5-p16 | GGSLTRTPLMSQLIEKPV | <u>SEQ ID NO: 38</u> |
| RD1-ORF5-p17 | SQLIEKPVAPSVMPAAAA | <u>SEQ ID NO: 39</u> |
| RD1-ORF5-p18 | SVMPAAAAGSSATGGAAP | <u>SEQ ID NO: 40</u> |
| RD1-ORF5-p19 | ATGGAAPVGAGAMGGAQ | <u>SEQ ID NO: 41</u> |
| RD1-ORF5-p20 | AMGGAQSGGSTRPGLVA | <u>SEQ ID NO: 42</u> |
| RD1-ORF5-p21 | TRPGLVAPAPLAQEREED | <u>SEQ ID NO: 43</u> |
| RD1-ORF5-p22 | AQEREEDDEDDWDEEDDW | <u>SEQ ID NO: 44</u> |
| RD1-ORF5-p23 | MLWHAMPPELNTARLMAG | <u>SEQ ID NO: 45</u> |
| RD1-ORF5-p24 | ARLMAGAGPAPMLAAAAG | <u>SEQ ID NO: 46</u> |
| RD1-ORF5-p25 | PMLAAAAGWQTLAALDA | <u>SEQ ID NO: 47</u> |
| RD1-ORF5-p26 | TLAALDAQAVELTARLN | <u>SEQ ID NO: 48</u> |
| RD1-ORF5-p27 | VELTARLNSLGEAWTGGG | <u>SEQ ID NO: 49</u> |
| RD1-ORF5-p28 | GEAWTGGGSDKALAAATP | <u>SEQ ID NO: 50</u> |
| RD1-ORF5-p29 | KALAAATPMVWLQTAST | <u>SEQ ID NO: 51</u> |
| RD1-ORF5-p30 | VWLQTASTQAKTRAMQAT | <u>SEQ ID NO: 52</u> |
| RD1-ORF5-p31 | KTRAMQATAQAAAYTQAM | <u>SEQ ID NO: 53</u> |
| RD1-ORF5-p32 | AAYTQAMATTPSLPEIAA | <u>SEQ ID NO: 54</u> |
| RD1-ORF5-p36 | TPSLPEIAANHITQAVLT | <u>SEQ ID NO: 55</u> |
| RD1-ORF5-p33 | LPEIAANHITQAVLTATN | <u>SEQ ID NO: 56</u> |

RD1-ORF5-p34 VLTATNFFGINTIPIALT SEQ ID NO: 57

RD1-ORF5-p35 NTIPIALTEMDYFIRMWN SEQ ID NO: 58

Kindly amend the paragraph at page 39, lines 8 to 14, to read as follows:

For cloning of the proteins, the following gene specific primers were used:

Rv2653c:

PA2653c: 5'- CTGAGATCTTTGACCCACAAGCGCACTAAA (*Bgl*II) SEQ ID NO: 59.

PB2653c: 5'- CTCCCATGGTCACTGTTTCGCTGTCGGGTTC (*Nco*I) SEQ ID NO: 60.

Rv2654c:

PA2654c: 5'- CTGAGATCTATGAGCGGCCACGCGTTGGCT (*Bgl*II) SEQ ID NO: 61.

PB2654c: 5'- CTCCCATGGTCACGGCGGATCACCCCGGTC (*Nco*I) SEQ ID NO: 62.

Kindly amend the paragraph at page 43, line 18 to page 44, line 15, to read as follows:

Rv2653c PEPTIDES

| | |
|---------------------------------|----------------------|
| Rv2653c-p1: MTHKRTKRQPAIAAGLNA | <u>SEQ ID NO: 7</u> |
| Rv2653c-p2: AIAAGLNAPRRNRVGRQH | <u>SEQ ID NO: 8</u> |
| Rv2653c-p3: RNRVGRQHGWPAADVPSAE | <u>SEQ ID NO: 9</u> |
| Rv2653c-p4: PADVPSAEQRRARQRDL | <u>SEQ ID NO: 10</u> |
| Rv2653c-p5: RAQRQRDLEAIRRAYAEM | <u>SEQ ID NO: 11</u> |
| Rv2653c-p6: IRRAYAEMVATSHEIDDD | <u>SEQ ID NO: 12</u> |
| Rv2653c-p7: TSHEIDDDTAELALLSMH | <u>SEQ ID NO: 13</u> |
| Rv2653c-p8: ELALLSMHLDDDEQRRLEA | <u>SEQ ID NO: 14</u> |
| Rv2653c-p9: DEQRRLEAGMKLGWHPYH | <u>SEQ ID NO: 15</u> |
| Rv2653c-p10:MKLGWHPYHFPDEPDSKQ | <u>SEQ ID NO:16</u> |

Rv2654c PEPTIDES

| | |
|--------------------------------|----------------------|
| Rv2654c-p1: MSGHALAARTLLAAADEL | <u>SEQ ID NO: 17</u> |
| Rv2654c-p2: AADELVGGPPVEASAAAL | <u>SEQ ID NO: 18</u> |
| Rv2654c-p3: ASAAALAGDAAGAWRTAA | <u>SEQ ID NO: 19</u> |
| Rv2654c-p4: AWRTAAVELARALVRAVA | <u>SEQ ID NO: 20</u> |

Rv2654c-p5: LVRVAESHGVA AVLFAA SEQ ID NO: 21

Rv2654c-p6: VLFAATAAAAAVDRGDPP SEQ ID NO: 22

Kindly amend the paragraph at page 47, line 8 to 22, to read as follows:

The coding regions Rv2653c and Rv2654c was amplified by PCR using the following sets of primers:

Rv2653-F: GGGGACAAGTTTGTACAAAAAAGCAGGCTTA TTG ACC CAC AAG CGC
ACT AA SEQ ID NO: 63

Rv2653-R: GGGGACCACTTTGTACAAGAAAGCTGGGTCCTA CTG TTT GCT GTC GGG
TTC GT SEQ ID NO: 64

Rv2654-F: GGGGACAAGTTTGTACAAAAAAGCAGGCTTA AGC GGC CAC GCG TTG
GC SEQ ID NO: 65

Rv2654-R: GGGGACCACTTTGTACAAGAAAGCTGGGTCCTA CGG CGG ATC ACC CCG
GT SEQ ID NO: 66

PCR reactions were carried out using Platinum Tag DNA Polymerase (GIBCO BRL) in a 50 µl reaction volume containing 60 mM Tris-SO₄ (pH 8.9), 18 mM Ammonium Sulfate, 0.2 mM of each of the four nucleotides, 0.2 µM of each primer and 10 ng of *M. tuberculosis* H37Rv chromosomal DNA. The reaction mixtures were initially heated to 95° C for 5 min., followed by 35 cycles of: 95° C for 45 sec, 60° C for 45 sec and 72° C for 2 min. The amplification products were precipitated by PEG/MgCl₂, and dissolved in 50 µL TE buffer.

Kindly replace the previously filed sequence listing with the enclosed pages entitled --Sequence Listing--.